EPA Reviewer: Lisa Austin, Ph.D. **Signature:** Lisa Austin
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 7/30/09
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HED Executive Summary Cover for the attached OECD Formatted DATA EVALUATION RECORD

STUDY TYPE: 28-Day Oral Toxicity feeding-[rat]; OPPTS 870.3100 [§82-1a] (rodent); OECD 408.

PC CODE: 118203 **DP BARCODE:** D349929

TEST MATERIAL (PURITY): BAS 800 H (94.2%)

SYNONYMS: AC 433379; BASF Reg. No. 4054449, saflufenacil

CITATION: Kaspers, U., Strauss, V., Kaufmann, W. et. al. (2007) BAS 800 H – Range Finding Study in Wistar rat - Administration in the diet for 4 weeks. Experimental Toxicology and Ecology BASF Aktiengesellschaft, Ludwigshafen, 67056 Ludwigshafen, FGR. Laboratory report number 33S0414/01147, June 6, 2007. MRID 47128108. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products, Research Triangle Park, NC.

EXECUTIVE SUMMARY:

In a 28-day oral toxicity study (MRID 47128108) BAS 800 H (94.2% a.i., Lot # COD 000227) was administered to Wistar rats, 5/sex/dose in the diet at dose levels of 0, 50, 150, 450, 1350, or 4050 ppm (♂ = 0, 4.5, 13.4, 39.2, 117, 357; ♀ = 0, 5.0, 15.9, 43.6, 130, 376, respectively).

There were no treatment-related findings in males at ≤150 ppm and in females at ≤450 ppm. Motor activity and functional observatory battery parameters were not affected in males and females. Clinical signs of toxicity observed in males at ≥1350 ppm were urine staining of anogenital regions and paleness of skin. Urine staining of anogenital areas was also evident in females at 4050 ppm.

The red blood cell (anemia), liver (extramedullary hemopoiesis), spleen (marked extramedullary hemopoiesis) and bone marrow (erythroid hyperplasia) were the targets. In males at 450 ppm, lower hemoglobin (Hb, 11%), mean corpuscular volume (MCV, 12%), and mean corpuscular hemoglobin (MCH, 15%) values were the only treatment-related findings.

At 1350 and 4050 ppm, BAS 800 H caused signs of general systemic toxicity, anemia and porphyria in both sexes, as well as decreased body weight (4-14%), body-weight gain (11-25%), and food consumption (8-17%) in the male. The following hematological parameters were significantly decreased: erythrocytes (RBC, 15-31%), hemoglobin (Hb, 9-43%), hematocrit (Hct, 6-36%), mean corpuscular volume (MCV, 7-13%), mean corpuscular hemoglobin (MCH, 10-

23%) and mean corpuscular hemoglobin concentration (MCHC, 10-11%). Reticulocytes (2-90%), white blood cells (WBC, 132-230%), lymphocytes (138-231%), and neutrophils (232-310) were significantly increased. It was stated in the study report that anisocytosis, and/or polychromasia were observed. Gross examination revealed an enlarged spleen in males at 1350 and 4050 ppm and in females at 4050 ppm. Absolute and relative spleen weights were significantly increased at >1350 ppm in both sexes (203-313%). In the liver and spleen of males at 1350 and 4050 ppm and females at 4050 ppm, extramedullary hematopoiesis (minimal to marked in severity) was evident. Erythroid hyperplasia was the microscopic finding in the bone marrow of males (4-5/5 vs 0 controls) at 1350 and 4050 ppm and of females (4/5 vs 0 in controls) at 4050 ppm.

The LOAEL established in males was 450 ppm (39.2 mg/kg bw/d) based on decreased Hb, MCV, and MCH. Increased polychromasia and anisocytosis were also observed at this dose. The NOAEL in males was 150 ppm (13.4 mg/kg bw/d). The LOAEL established in females was 1350 ppm (130.4 mg/kg bw/d) based on decreased Hb, hematocrit, MCV, and MCH. The NOAEL in females was 450 ppm (43.6 mg/kg bw/d).

This 28-day oral toxicity study in rats is acceptable, non-guideline; range-finding study and satisfies does not satisfy the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in mice.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging and Data Confidentiality statements were provided.

This Executive Summary was prepared for the United States Environmental Protection Agency, Office of Pesticide Program, Health Effects Division Use.

Much of the text was generated by the submitter(s) in OECD format. However, this document has undergone critical scientific analysis in comparison to the study report and modified as needed.

Reviewer #: Steve Wong, Ph.D., Date: April 21, 2008

APLICANT: BASF Corporation

STUDY TYPE: Short-term (28-day) dietary toxicity study in rat; OECD 407.

TEST MATERIAL (PURITY): BAS 800 H (94.2%)

SYNONYMS: AC 433379; BASF Reg. No. 4054449

CITATION: Kaspers, U., Strauss, V., Kaufmann, W. et al. (2007) BAS 800 H - Range finding study in Wistar rat; Administration in the diet over 4 weeks. Experimental Toxicology and Ecology, BASF Aktiengesellschaft 67056 Ludwigshafen, FGR. Report Number(s) 33S0414/01147. BASF Doc ID 2007/1028946. June 6, 2007. Unpublished. [PMRA # 1546987]

SPONSOR: BASF Corporation, Agricultural Products, Research Triangle Park, NC 27709

EXECUTIVE SUMMARY:

In a 28-day toxicity study, BAS 800 H (94.2% purity) was administered daily in the diet to Wistar rats, 5/sex/dose, at 0, 50, 150, 450, 1350, or 4050 ppm (♂ = 0, 4.5, 13.4, 39.2, 117, 357; ♀ = 0, 5.0, 15.9, 43.6, 130, 376, respectively). There were no treatment-related findings in males at ≤150 ppm and in females at ≤450 ppm. Motor activity and functional observatory battery parameters were not affected in males and females. Clinical signs of toxicity observed in males at ≥1350 ppm were urine staining of anogenital regions and paleness of skin. Urine staining of anogenital areas was also evident in females at 4050 ppm. At 1350 and 4050 ppm, BAS 800 H caused signs of general systemic toxicity, anemia and porphyria in both sexes, as well as decreased body weight, body-weight gain, and food consumption in the male. In males at 450 ppm, lower hemoglobin (Hb), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) values were the only treatment-related findings. The red blood cell (anemia), liver (extramedullary hemopoiesis), spleen (marked extramedullary hemopoiesis) and bone marrow (erythroid hyperplasia) were the targets. The LOAEL established in males was 450 ppm (39.2 mg/kg bw/d) based on decreased Hb, MCV, and MCH. Increased polychromasia and anisocytosis were also observed at this dose. The NOAEL in males was 150 ppm (13.4 mg/kg bw/d). The LOAEL established in females was 1350 ppm (130.4 mg/kg bw/d) based on decreased Hb, hematocrit, MCV, and MCH. The NOAEL in females was 450 ppm (43.6 mg/kg bw/d).

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

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I. MATERIALS AND METHODS

A. MATERIALS:

1.

Test material:	BAS 800 H (N'-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydro-1(2H)-pyrimidinyl)benzoyl]-N-isopropyl-N-methylsulfamide)
Description:	Solid crystalline / bright-beige; stored at room temperature
Lot/Batch #:	COD-000227
Purity:	94.2% a.i.
Compound stability:	The stability under the storage conditions present in this study was guaranteed by the Certificate of Analysis. The homogeneity of the test material was confirmed by analysis.
CAS #:	372137-35-4

2. **Vehicle and/or positive control:** The test substance was administered in the diet.

3. **Test animals:**

Species:	Rat	
Strain:	CrlGlxBrlHan:WI	
Age/weight at study initiation:	Age: ♂ = 34±1; ♀ = 33±1 day Mean weight: ♂ = 155. 5; ♀ = 122.8 g	
Source:	Charles River, Germany	
Housing:	Singly in DK III stainless steel wire mesh cages (floor area about 800 cm^2)	
Diet:	Kliba maintenance diet mouse/rat "GLP", meal, supplied by Provimi Kliba SA, Kaiseraugst, Switzerland; *ad libitum*	
Water:	Tap water *ad libitum*	
Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	20-24°C 30-70% 10/h 12h dark / 12h light
Acclimation period:	At least five days prior to application	

B. STUDY DESIGN:

1. **In life dates:** Start: June 16, 2004 End: July 16, 2004

2. **Animal assignment:** Animals were assigned to test groups via a randomization protocol provided by a computer. The test groups are noted in Table 1.

Table 1: Study design

	♂						♀					
ppm	0	50	150	450	1350	4050	0	50	150	450	1350	4050
mg/kg bw/d	0	4.5	13.4	39.2	117	357	0	5.0	15.0	43.6	130	376
N	5	5	5	5	5	5	5	5	5	5	5	5

3. Diet preparation and analysis:

For each concentration, BAS 800 H was weighed out and mixed with a small amount of food. Then appropriate amounts of food, depending on dose group, were added to this premix to obtain the desired concentrations. The BAS 800 H preparations were mixed once before the study.

The stability of the test substance in the diet was proven with a comparable batch of test substance for a period of up to 49 days at room temperature.

Before the start of the administration period, three samples were removed from the lowest and highest concentrations to verify the concentration and homogeneity of the test substance. A single sample was removed from the intermediate doses for concentration verification.

Results

Homogeneity analysis: For the 50 ppm dose preparation, the actual concentration was 95.7±2.2% (N=3) of the nominal concentration. For the 4050 ppm dose preparation, the actual concentration was 103.1±0.6% of the nominal concentration.

Stability analysis: In feed, BAS 800 H was stable for a period of 49 days (0 D: 100.0% of nominal; 9 D: 102.7% of nominal; 34 D: 95.3% of nominal; 49 D: 97.9% of nominal).

Concentration analysis: Mean analytical concentrations ranged from 95.7% to 103.1% of the nominal concentrations.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

4. Statistics:

Parameter	Statistical test[1]	References
Food consumption, body weight, body weight change, food efficiency	A comparison of each group with the control group using the Dunnett-test (2-sided) for the hypothesis of equal means	Dunnett, C.W. (1955): A multiple comparison procedure for comparing several treatments with a control. JASA, Vol. 50, 1096-1121 Dunnett, C.W. (1964). New tables for multiple comparisons with a control. Biometrics, Vol. 20, 482-491
Feces, rearing, grip strength length (forelimbs and hind limbs), foot-splay test, motor activity Clinical pathology parameters, except reticulo-cytes and differential blood count	Non-parametric one-way analysis using Kruskal-Wallis test (2-sided). If the resulting p ≤0.05, a pair-wise comparison of each dose group with the control group was performed using Wilcoxon-test (2-sided) for the equal medians	Siegel S. (1956): Non-parametric statistics for behavioral sciences. McGraw-Hill New York
Weight parameters	Non-parametric one-way analysis using Kruskal-Walis-test (2-sided). If the resulting p ≤0.05, a pairwise comparison of each dose group with the control group was performed using the Wilcoxon-test (2-sided) for the equal medians	Miller, R. G. (1981): Simultaneous Statistical Inference Springer-Verlag New York Inc., 165-167. International Mathematical and Statistical Libraries, Inc., 2500 Park West Tower One, Houston, Texas 77042-3020, USA, nakl-1-nakl-3. Nijenhuis, A. and Wilf, H.S. (1978): Combinatorial Algorithms, Academic Press, New York, 32-33. Hettmannsperger, T. P. (1984): Statistical Inference based on Ranks, John Wiley & Sons New York, 132-140.

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Urinalysis, except volume, color, turbidity and specific gravity	Pair-wise comparison of each dose group with the control group using Fisher's exact test for the hypothesis of equal proportions	Siegel S. (1956): Non-parametric statistics for behavioral sciences. McGraw-Hill New York

* Significantly different ($p < 0.05$) from the control. ** Significantly different ($p < 0.01$) from the control.

C. METHODS

1. Observations:

The rats were examined for signs of toxicity or mortality twice a day on weekdays and once a day on Saturdays, Sundays, and public holidays.

Detailed clinical observations (DCO)
Detailed clinical observations were conducted for all animals prior to the administration period and thereafter at weekly intervals. Parameters examined were as follows:

Skin / fur	Respiration	Urine	Pupil size	Palpebral closure
Posture	Tremors	Lacrimation	Impairment of gait	Activity / arousal level
Salivation	Convulsions	Exophthalmus	Abnormal movements	Feces (appearance/consistency)
Abnormal behaviour during handling				

Functional Observational battery (FOB)
A FOB was conducted for all rats at the end of the administration period.

Home cage observations

posture	tremor	convulsions	abnormal movements	impairment of gait	other findings

Open field observations

Fur / Skin	Lacrimation	Posture	Nose discharge	Abnormal movements
Salivation	Eyes/pupil size	Respiration	Impairment of gait	Feces within two minutes
Tremors	Palpebral closure	Convulsions	Activity/arousal level	Urine within two minutes
Behaviour when removed from cage			Number of rearings within two minutes	

Sensorimotor tests/reflexes

Vision	Approach response	Audition (startle response)	Landing foot-splay test
Papillary reflex	Touch response	Pain perception (tail pinch)	Other findings
Vocalization	Behaviour during handling	Coordination (righting response)	
Pinna reflex	Grip strength – hind limbs	Grip strength – forelimbs	

Motor activity assessment

Motor activity was measured on the same day as the FOB. Motor activity was assessed for 60 minutes in the dark using a Multi-Varimex-System (Columbus Instruments Int. Corp., USA).

2. Body weight:

Body weight was determined before the start of the administration period in order to randomize the animals. The weights were then determined on day 0 and weekly thereafter.

3. Food and water consumption and compound intake:

Food consumption for each rat was determined and mean daily diet intake was calculated as g food/kg bw/d. Food efficiency (body weight gain in g/food consumption in g per unit time X 100) and compound

intake (mg/kg bw/d) values were calculated as time-weighted averages from the food consumption and body weight gain data. Water consumption was observed daily by visual inspection of the water bottles for any overt changes in volume.

4. Ophthalmoscopic examination:

Eyes of all rats were examined on day 0 using an ophthalmoscope; those of control and high-dose rats were also examined on 23 (♂), and 22 (♀).

5. Hematology & clinical chemistry:

Blood was collected from fasted animals from the retro-orbital venous plexus or after decapitation. Hematological and clinical chemistry parameters were evaluated for all rats. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (Hct)*	X	Leukocyte differential count*
X	Hemoglobin (Hb)*	X	Mean corpuscular Hb (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular Hb concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Blood clotting measurements*	X	Reticulocyte count
X	Platelet count		
X	Clotting parameters - Prothrombin time (Thromboplastin time, Clotting time)		

* Recommended for subchronic rodent studies based on OECD 407 and EPA Guideline 870.3100

b. Clinical chemistry:

ELECTROLYTES				ENZYMES	
X	calcium*	X	chloride*	X	alkaline phosphatase (AP)
X	phosphorus*	X	magnesium		cholinesterase (ChE)
X	potassium*	X	sodium*	X	creatine phosphokinase
OTHERS					lactic acid dehydrogenase (LDH)
X	albumin*		blood creatinine*	X	alanine amino-transferase (ALT/SGPT)*
X	globulins	X	total bilirubin*	X	aspartate amino-transferase (AST/SGOT)*
X	glucose*	X	blood urea nitrogen*	X	gamma glutamyl transferase (GGT)*
X	total cholesterol	X	total serum protein (TP)*		glutamate dehydrogenase
X	triglycerides	X	total triiodothyronine (T3)	X	cyanide-insensitive palmitoyl-CoA-oxidation
X	total thyroxine (T4)	X	thyroid stimulating hormone (TSH)		

* Recommended for subchronic rodent studies based on OECD 407 and EPA Guideline 870.3100

6. Urinalysis:

The following parameters were analyzed in urine from all study animals on day 25 (♀) or 26 (♂).

x	volume	x	color	x	specific gravity	x	urobilinogen	x	protein	x	bilirubin
x	pH	x	turbidity	x	glucose	x	blood	x	ketones	x	sediment

7. Sacrifice and pathology:

All rats that died and those sacrificed at study termination were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs were weighed. The liver, spleen, bone marrow and adrenals (males only) were examined histopathologically in all dose groups. Other tissues were examined only in the control and the high-dose groups unless further histopathological examinations of other dose groups were necessary to define a NOAEL.

Digestive system				Cardiovacular/Hematologic				Neurologic system			
	Tongue				Aorta	xx	Spleen*	xx	Brain		Pituitary
	Salivary glands			xx	Heart*	xx	Thymus	x	Peripheral nerve		
	Esophagus			x	Bone marrow			x	Spinal cord (3 levels)		
x	Stomach			x	Lymph nodes				Eyes (optic nerve)		
x	Duodenum				**Urogenital system**				**Glandular organs**		
x	Jejunum	x	Colon	xx	Kidneys*+			xx	Adrenal gland*+		
x	Ileum	x	Rectum		Urinary bladder			x	Parathyroid	x	Thyroid
x	Cecum		Pancreas	xx	Testes+				Lacrimal gland		
xx	Liver*+			xx	Epididymides				Mammary gland		
x	Gall bladder			x	Prostate				**Other**		
	Respiratory system			x	Seminal vesicles			x	Bone		Skin
x	Trachea		Nose	xx	Ovaries				Skeletal muscle		
x	Lungs		Larynx	xx	Uterus & vagina			x	All gross lesions and masses*		
	Pharynx				Oviducts			x	Target Organs*		
								xx	Anesthetised animals		

* Recommended for subchronic rodent studies based on OECD 407 and EPA Guideline 870.3100
\+ Organ weights required.

II. RESULTS

A. Observations:

1. Clinical signs of toxicity:

Several males at 450 ppm and all animals at 1350 and 4050 ppm showed dark, discoloured urine. The discoloration of the urine was considered a compensatory response to the altered porphyrin metabolism. This response is treatment-related, but not adverse.

Skin paleness was observed in all rats at 1350 and 4050 ppm. Urine smeared anogenital region was observed with all male and three females at 4050 ppm and one male at 1350 ppm. One high-dose male showed a skin lesion that was judged to be treatment-related.

2. Mortality: No animals died prematurely.

B. Body weight and weight gain: Table 2

There were statistically significant decreases in body weight and body weight gain in males at 1350 and 4050 ppm. Body weights and body-weight changes in females were not affected.

Table 2: Body weight and body-weight gain in males, g±SD

	♂ (N = 5/group)					
ppm	0	50	150	450	1350	4050
mg/kg bw/d	0	4.5	13.4	39.2	117	357
Day 0	154.4±9.3	153.4±8.5	159.2±6.9	152.9±6.5	156.1±5.5	156.7±6.0
Day 7	201.9±11.4	199.3±11.5	207.0±13.7	194.6±9.1	193.5±8.4 (-4.2%)	193.5±7.8 (-4.2%)
Day 14	248.3±12.0	246.3±14.7	254.2±18.0	238.2±10.8	233.1±5.1 (-6.3%)	227.4±10.8 (-8.6%)
Day 21	286.7±11.9	282.9±20.4	286.2±23.5	268.8±11.8	262.6±7.9 (-8.4%)	**247.5±12.0** (-13.7%)**
Day 28	305.6±10.2	304.7±20.7	314.0±28.2	286.6±17.9	**271.7±6.1* (-11.1%)**	**269.9±21.8* (-11.7%)**
Body-weight gain, day 0-28	151.2±6.4	151.3±14.0	154.8±22.5	133.7±13.4 (-11.6%)	**115.6±10.7 (-23.5%)**	**113.2±16.9** (-25.1%)**

Data taken from pages 73-76 of Report; * p ≤0.05, ** p ≤0.01; **bolded** values are considered adverse treatment-related findings

C. Food consumption and compound intake:

1. Food consumption and food efficiency: Table 3

Food consumption was reduced in males at 1350 and 4050 ppm throughout most of the study period. Food efficiency in males at 4050 ppm was statistically significantly decreased on days 7, 14, and 21 and at 450 and 1350 ppm. The decrease at the 450 ppm was marginally lower than the control value and might not be treatment-related. There was no treatment-related effect on food consumption and food efficiency in females.

Table 3: Food consumption data of males, g of food/rat/d ± SD

	♂ (N = 5/group)					
ppm	0	50	150	450	1350	4050
mg/kg bw/d	0	4.5	13.4	39.2	117	357
	Food consumption (g/rat/d)					
Day 0	20.9±1.2	21.3±1.5	21.7±2.0	19.8±1.1	19.9±1.1	19.8±1.3
Day 14	23.9±0.9	23.8±1.9	24.3±2.1	22.1±1.1	21.9±1.1	**20.8±1.0***
Day 21	23.9±0.9	24.8±2.0	24.5±2.5	22.6±1.1	22.0±1.2	**21.1±0.9***
Day 28	21.5±0.9	21.7±1.7	22.3±2.3	19.9±1.3	**17.8±0.8****	20.0±2.0
	Food efficiency					
Day 7	32.6±0.8	30.8±0.9	31.3±1.8	30.0±0.9*	**26.7±1.4****	**26.6±1.8****
Day 14	28.6±2.6	28.3±1.9	27.8±1.5	28.2±2.4	25.8±4.2	**23.3±2.4***
Day 21	22.6±1.6	21.0±3.1	18.6±1.7	19.3±1.8	19.0±3.0	**13.7±3.1****
Day 28	12.6±3.1	14.4±1.7	17.7±2.8	12.6±4.3	7.3±3.5	15.6±6.0

Data taken from pages 71-72, 77-78 of Report; * p ≤0.05, ** p ≤0.01; bolded values are considered adverse treatment-related findings

2. Compound consumption: See Table 1 for the mean daily test substance intake in mg/kg bw/d.

D. Ophthalmoscopic examination: There were no treatment-related findings.

E. Blood analyses:

1. Hematology: Table 4

Dietary exposure to BAS 800 H at 1350 and 4050 ppm resulted in changes in several hematological parameters in both sexes. The affected parameters were erythrocytes (RBC), haemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), reticulocytes, white blood cells (WBC), lymphocytes, neutrophils, anisocytosis, and/or polychromasia (stated in study report, data not provided). In males at 450 ppm, statistically significantly lower Hb, MCV, and MCH were also reported.

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In the differential blood count the increases in leukocytes correlated with increases in lymphocytes, which were measured with an automated hematology analyzer using the principle of cytochemistry coupled with flow cytometry. Qualitative microscopic examination of blood smears of the high-dose rats, however, revealed no increases in lymphocytes. The increases in white blood cell counts were caused by an increase of normoblasts. The automated hematology analyzer could not distinguish between these abnormal cells and lymphocytes. As such, the increased white blood cell counts and lymphocytes are viewed as an analytical artifact.

Table 4. Selected hematological values, mean±SD

	♂ (N = 5/group)						♀ (N = 5/group)					
ppm	0	50	150	450	1350	4050	0	50	150	450	1350	4050
mg/kg bw/d	0	4.5	13.4	39.2	117	357	0	5.0	15.0	43.6	130	376
RBC, 10^{12}/L	7.83 ±0.60	8.04 ±0.17	7.76 ±0.50	8.25 ±0.72	**6.14** ±0.61**	**6.67** ±0.54**	8.01 ±0.15	7.95 ±0.37	7.77 ±0.38	7.59 ±0.45	8.18 ±0.43	**5.52** ±0.93**
Hb, mmol/L	9.4 ±0.2	9.6 ±0.1	9.2 ±0.3	**8.4** ±0.5**	**5.7** ±0.5**	**6.5** ±0.6**	9.3 ±0.2	9.3 ±0.3	9.0 ±0.4	9.2 ±0.3	**8.5* ±0.3**	**5.3** ±0.6**
Hct, %	43.1 ±2.0	44.4 ±0.9	41.8 ±2.0	40.0 ±2.2	**29.4** ±2.8**	**33.2** ±2.2**	42.6 ±1.0	42.2 ±1.8	40.7 ±2.2	41.4 ±2.2	**40.1* ±1.4**	**27.3** ±4.0**
MCV, fL	55.1 ±2.5	55.3 ±0.7	53.9 ±2.4	**48.6* ±2.6**	**47.9* ±1.1**	**49.9* ±1.3**	53.3 ±1.0	53.1 ±2.0	52.4 ±1.3	54.6 ±1.7	**49.1** ±1.3**	**49.6** ±1.6**
MCH, fmol	1.20 ±0.06	1.19 ±0.02	1.19 ±0.07	**1.02* ±0.09**	**0.92** ±0.04**	**0.97** ±0.04**	1.16 ±0.02	1.17 ±0.07	1.15 ±0.03	1.21 ±0.05	**1.04** ±0.04**	**0.97** ±0.07**
MCHC, mmol/L	21.8 ±0.63	21.6 ±0.38	22.0 ±0.67	21.1 ±0.75	**19.3** ±0.44**	**19.5** ±0.52**	21.7 ±0.59	22.1 ±0.19	22.0 ±0.19	22.2 ±0.47	21.3 ±0.25	**19.6** ±1.05**
WBC, 10^9/L	5.42 ±1.06	5.59 ±0.68	6.15 ±1.24	7.00 ±1.48	**16.24** ±2.66**	**12.56** ±2.81**	5.57 ±2.03	5.56 ±1.13	6.27 ±1.08	5.21 ±0.58	5.39 ±1.41	**18.36** ±1.75**
Neutrophils 10^9/L	0.75 ±0.09	0.70 ±0.18	0.70 ±0.14	1.11 ±0.28	**2.89 ±0.72**	**1.74 ±0.36**	0.52 ±0.23	0.38 ±0.06	0.80 ±0.56	0.45 ±0.07	0.59 ±0.41	**2.13 ±0.51**
Lymphocyte 10^9/L	4.42 ±1.03	4.61 ±0.71	5.19 ±1.06	5.64 ±1.29	**12.99 ±2.56**	**10.53 ±3.02**	4.77 ±1.74	4.89 ±1.04	5.20 ±1.06	4.56 ±0.56	4.60 ±1.20	**15.81 ±2.06**
Platelets, 10^9/L	1079 ±134	1021 ±144	986 ±61	1027 ±147	**1384* ±166**	1104 ±132	895 ±231	1029 ±88	1025 ±61	986 ±153	950 ±133	**1704** ±97**
Reticulocyte %	2.1 ±0.4	2.1 ±0.3	2.0 ±0.4	2.7 ±0.5	4.0 ±0.9	7.6 ±1.9	2.2 ±0.5	1.8 ±0.2	2.1 ±0.3	1.8 ±0.5	2.6 ±0.4	7.9 ±2.8

Data taken from Table IB, pages 107-122 of Report; * ≤0.05; ** ≤0.01; bold values are considered treatment-related

2. Clinical chemistry: Table 5

Table 5. Selected clinical chemistry and hormone values, mean±SD

	♂ (N = 5/group)						♀ (N = 5/group)					
ppm	0	50	150	450	1350	4050	0	50	150	450	1350	4050
mg/kg bw/d	0	4.5	13.4	39.2	117	357	0	5.0	15.0	43.6	130	376
T protein g/L	63.7 ±1.90	64.8 ±1.21	63.5 ±2.33	62.1 ±1.42	61.2 ±1.16	**58.1* ±1.31**	63.5 ±2.54	64.9 ±2.42	61.6 ±3.21	64.4 ±3.66	65.5 ±2.12	**59.1** ±1.80**
Albumin g/L	37.1 ±1.18	37.8 ±0.99	37.0 ±1.20	36.6 ±0.82	36.5 ±0.74	**35.4* ±0.73**	38.5 ±1.79	39.2 ±1.45	37.0 ±2.27	39.0 ±1.96	39.8 ±1.05	**36.0* ±0.98**
Globulin g/L	26.6 ±0.84	26.9 ±0.36	26.5 ±1.19	25.5 ±0.94	**24.7* ±0.79**	**22.7** ±1.13**	25.1 ±0.08	25.7 ±1.42	24.7 ±2.27	25.4 ±1.92	25.7 ±1.25	23.1 ±1.05
Bilirubin µmol/L	2.05 ±0.37	2.07 ±0.26	1.89 ±0.41	2.42 ±0.35	**3.60** ±0.33**	**4.50** ±1.24**	2.79 ±0.35	2.63 ±0.45	2.57 ±0.62	2.56 ±0.29	**2.26* ±0.28**	**3.80** ±0.46**
T3 nmol/L	0.93 ±0.26	0.85 ±0.15	0.85 ±0.11	0.79 ±0.07	0.80 ±0.21	0.73 ±0.09	0.75 ±0.19	0.83 ±0.16	0.72 ±0.07	0.83 ±0.07	**1.00* ±0.12**	0.63 ±0.14
T4 nmol/L	47.8 ±6.44	40.3 ±4.57	39.9 ±9.63	40.4 ±3.21	38.5 ±6.71	31.1 ±7.54	22.9 ±7.13	25.2 ±1.87	23.8 ±4.42	21.3 ±2.42	**36.0* ±5.47**	20.9 ±4.95

Data taken from Table IB, pages 107-122 of Report; * ≤0.05; ** ≤0.01; bold values are considered treatment-related

Compound-related differences in serum enzyme activities were not evident at any dose level in either males or females.

Blood chemistry examinations revealed statistically significantly decreased total protein, albumin and globulin concentrations and increased total bilirubin levels in the males at 4050 ppm and reduced globulin concentrations as well as high total bilirubin concentrations in the serum of the males at 1350 ppm. Similar decreases in total protein, albumin and globulin levels as well as increases in total bilirubin concentration were observed in the females at 4050 ppm. With the exception of the increase in total bilirubin values, the magnitude of changes in most of these values was small and could represent normal biological variations.

3. Hormones:

No treatment-related effects on the serum hormone levels (T3, T4, TSH) were found in either sex, although the T3 and T4 levels in the high-dose males appeared to be lower than the control values.

F. Urinalysis:

Urine specimens of the males at ≥450 ppm and of the females at 4050 ppm were discoloured from light yellow orange to maize yellow. In addition, urinalyses revealed significantly increased urobilinogen levels in the males at ≥150 ppm (4-5/5 vs 0/5 controls) and in the females at 4050 ppm (5/5 vs 1/5 controls). Slightly, but not statistically significantly increased urinary urobilinogen concentrations were also found in the females at 1350 ppm. No treatment-related effects were seen in the other urine parameters.

G. Sacrifice and pathology:

1. Organ weight:

The only treatment-related findings were the increases in absolute and relative spleen weights in males at 1350 and 4050 ppm, and in females at 4050 ppm. The increase was associated with significant extramedullary hematopoiesis in the spleen. Other statistically significant findings, for example, adrenal weights, might be secondary to significant decreases in terminal body weights. Because of the inconsistencies of the findings, the lack of a dose response, the absence of pathomorphological correlates, and the small magnitude of the changes, these findings were considered to be of no biological significance.

Table 6. Selected absolute and relative spleen weight values (mean±SD)

	♂ (N = 5/group)						♀ (N = 5/group)					
ppm	0	50	150	450	1350	4050	0	50	150	450	1350	4050
mg/kg bw/d	0	4.5	13.4	39.2	117	357	0	5.0	15.0	43.6	130	376
BW, g	274.0 ±12.6	270.2 ±18.2	277.3 ±25.5	255.4 ±13.6	**243.1** ±8.6**	**239.3** ±14.6**	158.2 ±8.4	172.5 ±7.4	170.6 ±7.2	163.9 ±6.8	168.7 ±9.4	161.5 ±11.1
Spleen mg	506 ±59	514 ±91	542 ±56	544 ±105	**1532** ±349**	**1766** ±733**	338 ±63	362 ±53	424 ±35	364 ±25	404 ±65	**1432** ±38**
%	0.184 ±0.018	0.189 ±0.023	0.196 ±0.020	0.212 ±0.033	**0.630** ±0.138**	**0.735** ±0.288**	0.213 ±0.034	0.210 ±0.029	0.249 ±0.018	0.222 ±0.017	0.238 ±0.026	**0.880** ±0.195**
Adrenal mg	75.0 ±5.1	73.4 ±8.4	65.6 ±3.5*	59.0 ±5.1**	59.6 ±4.9**	58.8 ±1.3**	75.2 ±8.4	70.0 ±7.5	77.4 ±10.4	67.8 ±10.5	72.6 ±6.6	63.0 ±7.2
%	0.027 ±0.003	0.027 ±0.003	0.024** ±0.001	0.023** ±0.002	0.025 ±0.002	0.025 ±0.002	0.047 ±0.005	0.041 ±0.003	0.045 ±0.005	0.042 ±0.007	0.043 ±0.006	0.039 ±0.006
Data taken from Table IC. pages 127-130 of Report; * ≤0.05; ** ≤0.01; bold values are considered treatment-related												

2. Gross pathology:

The only treatment-related effect was the enlarged spleens in males at 1350 and 4050 ppm and in females at 4050 ppm.

3. Microscopic pathology: Table 7

Substance-induced findings were observed in the liver, spleen, and bone marrow. In the liver and spleen of males at 1350 and 4050 ppm and females at 4050 ppm, extramedullary hematopoiesis was evident. Erythroid hyperplasia was the microscopic finding in the bone marrow of males at 1350 and 4050 ppm and of females at 4050 ppm. The microscopic findings in these tissues were directly related to anemia and porphyria. All other findings noted were considered to be spontaneous or incidental in origin and not related to treatment.

Table 7. Selected microscopic findings (number of animals affected)

			♂ (N = 5/group)						♀ (N = 5/group)					
ppm			0	50	150	450	1350	4050	0	50	150	450	1350	4050
mg/kg bw/d			0	4.5	13.4	39.2	117	357	0	5.0	15.0	43.6	130	376
bone marrow	Erythroid hyperplasia					1	**3**	**4**			1			**4**
liver	Fatty change, diffuse,	gr 2						**1**						
	Focal necrosis							**2**		**1**		**1**	**1**	**1**
	Extramedullary hematopoiesis	gr 1 gr 2 gr 3					**1** **3** **1**	**2** **3**						 **3** **2**
spleen	Marked extramedullary hematopoiesis	gr 1 gr 2 gr 3 gr 4				**1**	 **5**	 **1** **4**	1 1		**1**	1 **1**	**2**	 **4** **1**
Data taken from Table IC, pages 133-139 of Report; * ≤0.05; ** ≤0.01; bold values are considered treatment-related gr = grade: 1 = minimal, 2 = slight, 3 = moderate, 4 = marked, 5 = extreme														

H. Functional observational battery (FOB):

Most findings were equally distributed between test and control groups, were without a dose-response relationship, or occurred in single animals only; these observations were considered as incidental.

Open field observations: Treatment-related findings were urine staining of the anogenital region and skin paleness (all rats at 4050 ppm). Paleness of the skin was also observed in males at 1350 ppm.

Sensorimotor tests/reflexes: No substance-related effects were observed.

I. Motor activity:

No significant deviations were seen in the treated males or females in the summation intervals. Isolated statistically significant findings lacked of a dose response and the magnitude of the changes was low. The latter findings were not considered toxicologically relevant.

III. DISCUSSION

A. Authors' conclusions:

The oral administration of BAS 800 H in the diet to male and female rats over four weeks caused signs of general systemic toxicity, anemia and porphyria. The liver, spleen and bone marrow were recognized as targets of the test article, which is an inhibitor of protoporphyrinogen IX oxidase. The enzyme inhibition causes porphyria and anemia through blockage of the conversion of porphyrins into hemoglobin.

The LOAEL established in males was 450 ppm (39.2 mg/kg bw/d) based on decreased Hb, MCV, and MCH. Increased polychromasia and anisocytosis was also observed at this dose. The NOAEL in males was 150 ppm (13.4 mg/kg bw/d).

The LOAEL established in females was 1350 ppm (130.4 mg/kg bw/d) based on decreased Hb, Hct, MCV, and MCH. The NOAEL in females was 450 ppm (43.6 mg/kg bw/d).

A. Reviewer's comments:

The study was properly conducted and reported. The authors' conclusions are valid.